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Comparison of Traditional and Commercial Vinegars Based on Metabolite Profiling and Antioxidant Activity

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Metabolite profiles of seven commercial vinegars and two traditional vinegars were performed by gas chromatography time-of-flight mass spectrometry with multivariate statistical analysis. During alcohol fermentation, yeast, *nuruk*, and *koji* were used as sugars for nutrients and as fermentation substrates. Commercial and traditional vinegars were significantly separated in the principal component analysis and orthogonal partial least square discriminant analysis. Six sugars and sugar alcohols, three organic acids, and two other components were selected as different metabolites. Target analysis by ultra-performance liquid chromatography quadruple-time-of-flight mass spectrometry and liquid chromatography-ion trap-mass spectrometry/mass spectrometry were used to detect several metabolites having antioxidant activity, such as cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside, and quercetin, which were mainly detected in Rural Korean Black raspberry vinegar (RKB). These metabolites contributed to the highest antioxidant activity measured in RKB among the nine vinegars. This study revealed that MS-based metabolite profiling was useful in helping to understand the metabolite differences between commercial and traditional vinegars and to evaluate the association between active compounds of vinegar and antioxidant activity.

Keywords: Antioxidant activity, commercial vinegar, mass spectrometry, metabolite profiling, traditional vinegar

Introduction

Vinegar has been made from a variety of agricultural materials and has been used since around 3000 BC in Asian, European, and other traditional cuisines of the world [3, 8]. It is a traditional fermented food with a sour flavor and is widely used as an acidic seasoning, preservative, beverage, and dressing. It contains specific volatile and nonvolatile compounds, including organic acids, sugars, amino acids, and esters [10]. The health benefits of drinking vinegar are well known and include an immune modulation effect, resistance to cardiovascular disease, appetite suppression, improved digestion, decreased serum cholesterol and blood pressure [29], and reduced fasting blood glucose [11, 17].

According to the Korean food standard code, vinegar is divided into traditional vinegar and commercial vinegar [12]. The manufacturing methods involve the use of common materials that are rich in sugars or starches, such as fruits juices, that go through alcohol fermentation and oxidation of ethanol to acetic acid [20]. These fermentation processes are carried out using various kinds of microorganisms, including molds, yeasts, and bacteria. These organisms produce acetic acid, as well as various metabolic compounds that control the taste and flavor of vinegar [2]. However, differences in periods of fermentation, cost of production, and maintaining a constant quality have been issues between traditional and commercial vinegars [12, 10].

The influence of industrialization in the 1970s created a sharp increase in productivity and consumption of cheap commercial vinegars, which were a major product on the

market [10, 31]. During the 1990s, traditional vinegars gained popularity by many customers because of increased recognition of diet and pursuit of health enhancement, showing that traditional vinegars were better than commercial vinegars [7].

To reveal the effect and activity of vinegar, many studies have been performed comparing its antioxidant activity [2, 17], and measuring the content of sugars, organic acids, and amino acids [25, 10], and in *in vivo* studies, acute cardiovascular effects on rats [29] and inhibition of lipid peroxidation in mouse serum and liver [26] have been shown. On the other hand, using a metabolomics approach for vinegars, measuring the amount of volatile or nonvolatile metabolites for the development of flavors and taste connected with mass spectrometry (MS) technique was reported [20]. MS-based vinegar research uses mainly GC-MS analysis to detect volatile aroma compounds [20], and targeted analysis using high-performance liquid chromatography-photodiode array detector-MS (HPLC-DAD-MS) to detect phenolic compounds [24].

Metabolomics is a field that studies the change in the metabolites in various ecological conditions, and has been increasingly used to characterize the natural variance of target specimens and to compare the metabolic composition of primary and secondary metabolites in fermented foods, such as fermented soybean paste (*cheonggukjang*) [15], vinegars [22], cheeses [23], and Korean traditional *meju* [21]. However, the studies of non-target analysis for primary metabolite profiling of vinegars using gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) are less reported.

Therefore, the objectives of this study were metabolite profiling combined with MS techniques and multivariate analysis to characterize the metabolic differences between commercial and traditional vinegars and to evaluate their antioxidant activities.

Materials and Methods

Chemicals and Reagents

Methoxyamine hydrochloride and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA), used in derivation for GC-TOF-MS, were purchased from Sigma Aldrich (St. Louis, MO, USA). 2-2′-Azino*bis* (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium acetate, 2,4,6-tripyridinyl-s-triazine (TPTZ), ferric chloride hexahydrate, sodium hydroxide (NaOH), diethylene glycol, folin-ciacaltam, sodium carbonate ($NaCO_3$), and other standards (gallic acid, naringin, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)), which were used for total acidity, antioxidant assays (ABTS, DPPH, and FRAP), total flavonoid contents, and total polyphenol contents, were also purchased from Sigma Aldrich (USA).

Materials

Seven commercial vinegars were purchased from the open market in Seoul, Korea, and two traditional vinegars (rural Korean Black raspberry (*Rubus coreanus* Miquel) vinegar (RKB) and the rural detoxified lacquer (*Rhus verniciflua* stokes) vinegar (RLQ)) were provided by the Korean Rural Department Administration (Suwon, Korea). The details for each vinegar (major ingredients,

Table 1. Vinegar samples information.

Vinegar sample	Sample name	Resource	Major ingredients (%)	pH	Total acidity (%)
BS	Balsamic vinegar	Open market	Balsamic vinegar 35%, red grapes condensed matter 23%	2.91 ± 0.02	4.55 ± 0.05
JA	Japanese apricot vinegar	Open market	Japanese apricot condensed matter 5%, apple condensed matter 19.8%	3.21 ± 0.00	4.72 ± 0.06
YZ	Yuzu vinegar	Open market	Citron fruit juice 15.08%, alcohol	2.64 ± 0.01	4.60 ± 0.03
LM	Lemon vinegar	Open market	Lemon condensed matter 3.5%	2.65 ± 0.01	6.10 ± 0.06
AP	Apple vinegar	Open market	Apple condensed matter 5.6%	2.72 ± 0.01	5.98 ± 0.07
KB	Korean Black raspberry vinegar	Open market	Bokbunja 95%, apple condensed matter	3.19 ± 0.01	2.14 ± 0.03
BR	Brown vinegar	Open market	Brown rice sugar condensed matter 6.81% (Brown rice 99%)	2.59 ± 0.01	6.30 ± 0.03
RLQ	Rural lacquer vinegar	Rural Development Administration	*Rhus verniciflua* stokes 2%	2.99 ± 0.01	5.87 ± 0.03
RKB	Rural Korean Black raspberry vinegar	Rural Development Administration	*Rubus coreanus* Miquel 100%	3.01 ± 0.02	4.75 ± 0.05

All values are expressed as mean ± SD.

pH, total acidity, and full name of samples) are given in Table 1.

Fermentation Processes of RKB, RLQ, and Commercial Vinegar

In the RKB process (Fig. 1A), the initial conditions for alcohol fermentation were as follows: sugar concentration of 25°Brix, temperature of 25°C, and duration of 10 days. After alcohol fermentation, water was added to make 6% alcohol. The conditions for acetic acid fermentation were stationary fermentation for 2–3 weeks at 30°C with *jongcho*, which was made from Korean Black raspberry wine with *Acetobacter pasterius* RDAFR KACC16934, with a total acidity of 4%.

In the RLQ process (Fig. 1B), the alcohol fermentation of sticky rice and brown rice was performed using rice-*nuruk (Saccharomyces cerevisiae (La Parisienne))* and *koji* for 10 days at 20°C with addition of 5% rushiol-free fermented *Rhus verniciflua* strokes stem bark extracts. Acetic acid fermentation was sustained for 3–4 weeks at 30°C with *jongcho*, which was made from rushiol-free wine with *Acetobacter pasteurianus* CV3 KACC17058, with a total acidity of 4%. The commercial vinegar process (Fig. 1C) used edible alcohol with fruit squeeze instead of the alcohol fermentation process, followed by acetic acid fermentation with *Acetobacter* and some other artificial sweeteners.

Analysis of pH and Total Acidity

The pH of the vinegars was measured with a pH meter (Termo, USA). Analysis of total acidity was according to a slightly modified method of Jo *et al*. [12]. All vinegars were titrated using a 0.1 N NaOH solution and an endpoint of pH 8.2 for measuring total acidity.

The results for the pH and total acidity of the various vinegars are presented in Table 1. The pH values of the vinegars ranged from approximately 2.5 to 3.2, and total acidities were approximately 4%–6%, which were suitable according to the classification of the Korean food standard code [12]. All experiments were performed in triplicate.

Sample Preparation

Vinegar samples were mixed with an equal volume of a solvent (vinegar:methanol = 1:1 (v/v)) using a Twist Shaker (Biofree, Seoul, Korea) for 1 h, and centrifuged at 4°C and 5,000 rpm for 5 min (Hettich Zentrifugen, Universal 320R, Germany) with 50 ml centrifuge tubes. After centrifuging, supernatants were transferred to new tubes and completely dried in a speed vacuum concentrator (Biotron, Seoul, Korea). For GC-TOF-MS analysis, 50 µl of methoxyamine hydrochloride (2% in pyridine) was added to the

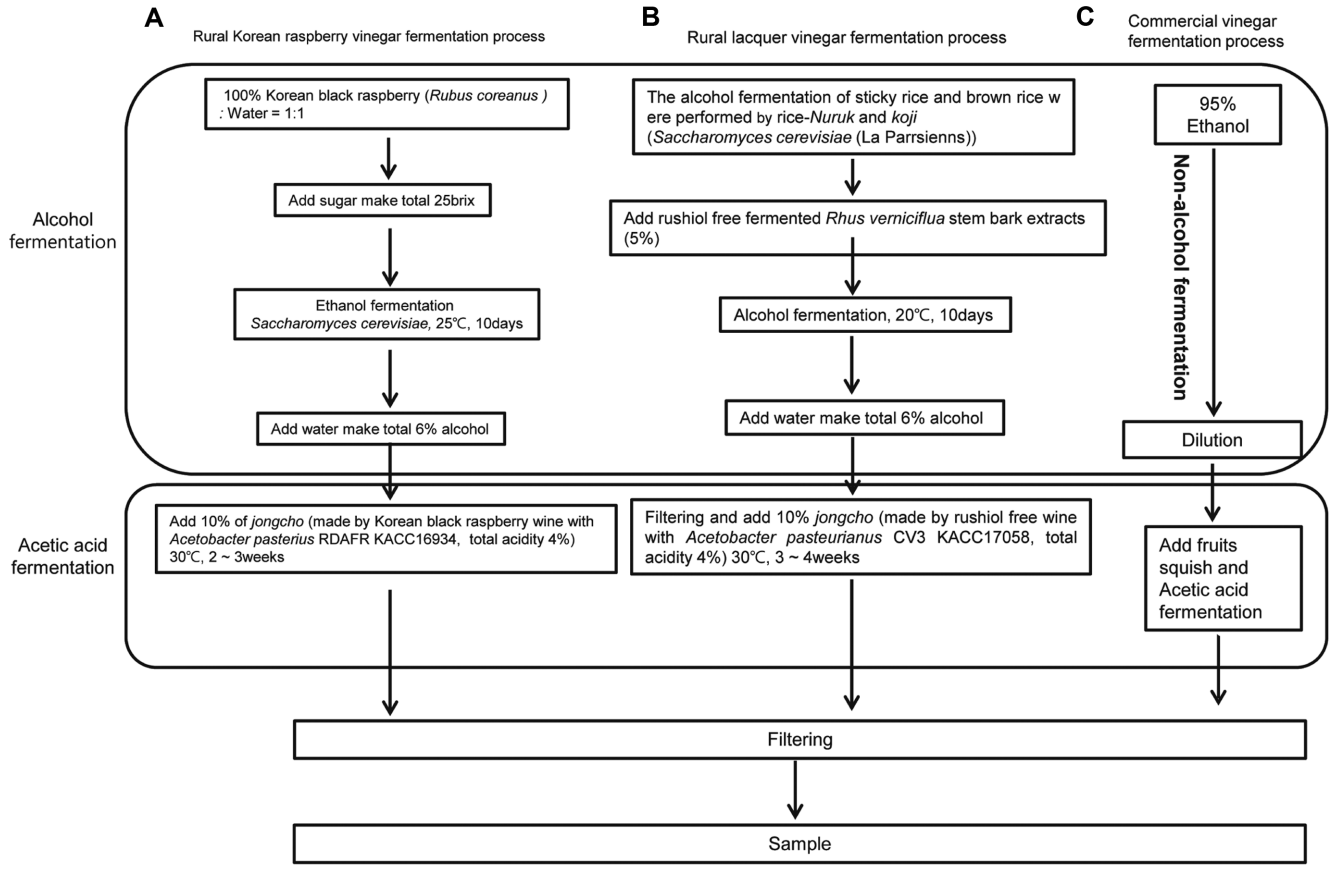

Fig. 1. Flow diagrams showing the fermentation process for Rural Korean Black raspberry vinegar (RKB) (**A**), Rural lacquer vinegar (RLQ) (**B**), and commercial vinegar (**C**).

extracted samples that were dissolved in 50% methanol and oximated at 30°C for 90 min. Then, the oximated samples were silylated with 50 µl of MSTFA at 37°C for 30 min. All derivatized samples were filtered through a 0.2 µm polytetrafluoroethylene (PTFE) filter. For target analysis, the dried samples were dissolved in 50% methanol and then filtered through a 0.2 µm PTFE filter. Three analytical replications from two different batches were used for both GC-TOF-MS and ultra-performance liquid chromatography quadruple-time-of-flight mass spectrometry (UPLC-Q-TOF-MS) analyses.

Analysis of Various Vinegar Compounds by GC-TOF-MS

For GC-TOF-MS analysis, each sample (1 µl) was injected into an Agilent 7890A gas chromatography system equipped with an Agilent 79693 autosampler (Agilent, Atlanta, GA, USA) coupled to a Pegasus Time of Flight-Mass Spectrometer detector (Leco, St. Joseph, MI, USA). Metabolites were separated on an RTx-5MS column (30 m × 0.25 mm; film thickness, 0.25 µm) and helium was used as the carrier gas at a constant flow rate of 1.5 ml/min. The temperature program for metabolome analysis started with a 2 min isothermal step at 75°C, followed by temperature ramping of 15°C to a final temperature of 300°C, which was maintained for 3 min. The acquisition rate was set to 30 spectra with the mass range of 45–1,000 *m/z*.

Target Analysis of the Metabolites Associated with Antioxidant Activity Using UPLC-Q-TOF-MS

Ultra-performance liquid chromatography-quadruple-time-of-flight mass spectrometry was used to analyze secondary metabolites. Extracted vinegars were analyzed using a Waters Micromass Q-TOF Premier UPLC-Q-TOF-MS system with a UPLC Acquity System (Waters, Milford, MA, USA). Analysis was performed with an Acquity UPLC BEH C18 column (100 × 2.1 mm; Waters; 1.7 µm particle size). The mobile phase consisted of A (0.1% (v/v) formic acid in water) and B (0.1% (v/v) formic acid in acetonitrile) and the gradient conditions were increased from 5% to 100% acetonitrile over 10 min, and then decreased back to 5% over 2 min. The flow rate was maintained at 0.3 ml/min and the volume of sample injected was 5 µl. ESI was performed in negative (–) and positive (+) ion modes within a range of 100–1,000 *m/z*. The operating parameters were as follows: ion source temperature, 100°C; cone gas flow, 0.0 l/h; desolvation gas flow, 650 l/h; capillary voltage, 2.5 kV; and cone voltage, up to 50 V. The UPLC-Q-TOF-MS raw data were analyzed using MassLynx software (Waters Corp.), which was used to calculate accurate masses and elemental compositions for the metabolite scans.

Target Analysis of the Metabolites Associated with Antioxidant Activity Using LC-IT-MS/MS

Liquid chromatography-ion trap-mass spectrometry/mass spectrometry (LC-IT-MS/MS) analysis was performed using a Varian 500-MS ion trap mass spectrometer (Varian, USA), which consisted of an LC pump (Varian 212), an auto sampler (Prostar 410), and a photodiode array detector (Prostar 335). The LC system was equipped with a Varian PurSuit XRs C18 column (i.d., 100 mm × 2.0 mm; 3 µm particle size; Varian, Lake Forest, CA, USA). The initial mobile phase conditions consisted of 90% A (0.1% (v/v) formic acid in water) and 10% B (0.1% (v/v) formic acid in acetonitrile), which was maintained for 2 min, followed by an increase to 90% B for 25 min. The gradient was maintained at 90% B for 5 min, rapidly decreased to 50% B for 0.06 min, and then maintained for 5 min. The flow rate was set to 0.2 ml/min and the volume of sample injected was 10 µl. The full-scan mass spectral range was 100–1,000 *m/z*. The operating parameters for analyzing the samples were as follows: spray needle voltage, 5 kV; capillary voltage, 80 V; drying temperature, 300°C; drying gas pressure (nitrogen), 20 psi; nebulizer gas pressure (air), 40 psi. Tandem mass spectrometry analysis was carried out using scan-type turbo data-dependent scanning (DDS) under the same conditions. LC-IT-MS/MS data were analyzed using the MS workstation software (ver. 6.9; Varian, USA).

Data Processing and Statistical Analysis

GC-TOF-MS raw data files were converted to computable document form (*.cdf) using the inbuilt data processing software of the Agilent GC system program. After acquiring the data in .cdf format, the files were subjected to preprocessing alignment by the metAlign software package (http://www.metalign.nl). After alignment, the resulting peak list was obtained as a .txt file, which was exported into Microsoft Excel (Microsoft, Redmond, WA, USA). The Excel file included the corrected peak retention times (min), peak areas, and corresponding mass (*m/z*) data for further analysis.

Primary metabolites were represented through multivariate statistical analysis using SIMCA-P$^+$ 12.0 software (Umerics, Umea, Sweden) to identify metabolite differences between commercial and traditional vinegars.

Orthogonal partial least square discriminant analysis (OPLS-DA) was used for the processing and classification of GC-TOF-MS data to identify metabolites that showed variations between commercial vinegars and traditional vinegars. All variables were selected based on the variable importance in projection values (VIP > 0.7) and significance ($p < 0.05$) for Student's t-test of individual samples, and the variable selection was used and compared by box-and-whisker plots using STATISTICA (ver. 7.0; Stat Soft, Tulsa, OK, USA). The variable selection was annotated based on standard retention time, *m/z*, and existing references.

Determination of Antioxidant Activity by ABTS, DPPH, and FRAP

The ABTS assay protocol followed the method of Re *et al*. [27] with slight modifications. The stock solution was 2.45 mM potassium persulfate solution with 7 mM ABTS. The solution was diluted until the absorbance reached 0.7 ± 0.02 at 750 nm using a spectrophotometer (SpectronicGenesys 6; Thermo Electron, Madison, WI, USA). Ten microliters of extracted vinegar was reacted with

190 µl of the diluted ABTS solution for 7 min in the dark, followed by absorbance measurement at 750 nm.

The DPPH assay protocol was conducted according to the method of Lee *et al*. [19] with slight modifications. Briefly, 20 µl of each vinegar extract was reacted with 180 µl of DPPH ethanol solution for 20 min at room temperature in the dark, followed by absorbance measurement at 515 nm.

The FRAP assay was conducted according to the method of Benzie and Strain *et al*. [1] with slight modifications. Briefly, the FRAP reagent was prepared by mixing acetate buffer (pH 3.6), 10 mM TPTZ (in 40 mM HCl solution), and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1. Then, 10 µl of each vinegar extract was reacted with 300 µl of FRAP reagent for 6 min at room temperature in the dark. The absorbance was measured at 570 nm. In all experiments, standard curves were linear between 0.0625 and 2 mM Trolox equivalents, and results were presented in µmol Trolox equivalents (TE) per gram sample extract. All experiments were performed in triplicate.

Determination of Total Polyphenol Contents (TPC) and Total Flavonoid Contents (TFC)

TPC was determined according to the method of Folin-Denis with slight modifications. Twenty microliters of each vinegars extract was reacted with 100 µl of 0.2 N folin-ciocaltam for 5 min in the dark. Then, 80 µl of 7.5% $NaCO_3$ was added, followed by incubation for 60 min in the dark, and absorbance measurement at 750 nm using a spectrophotometer. Standard curves were linear between 7.81 and 500 ppm gallic acid equivalents and results were presented in ppm gallic acid per gram sample extract.

The TFC protocol was conducted according to the method of Jung *et al*. [13] with slight modifications. Briefly, 20 µl of each vinegar extract was reacted with 20 µl of 1 N NaOH and 180 µl of 90% diethylene glycol for 60 min in the dark and absorbance was measured at 405 nm using a spectrophotometer. Standard curves were linear between 6.25 and 200 ppm naringin equivalents and results were presented in ppm naringin per gram sample extract. All experiments were performed in triplicate.

Results

Multivariate Analysis of Various Vinegars Performed on GC-TOF-MS Data

In Fig. 2A, the PCA model of seven commercial vinegars and two traditional vinegars (RKB and RLQ) were separated by PC3, and the scores explained 21.9% of the total variability (PC2: 12.6%; PC3: 9.3%).

The OPLS-DA (Fig. 2B) showed a clear difference between commercial and traditional vinegars. Significantly different primary metabolites were selected from OPLS-DA, based on VIP (VIP > 0.7) and *p*-values (*p*-value < 0.05), and the identified metabolites were annotated by their retention time compared with standard mass fragmentation (Table 2). Discriminated metabolites of commercial and traditional vinegars were presented by box-and-whisker plots, which were calculated by peak area (Fig. 3). Fructose, glucose, sorbitol, glucopyranose, malic acid, and citric acid

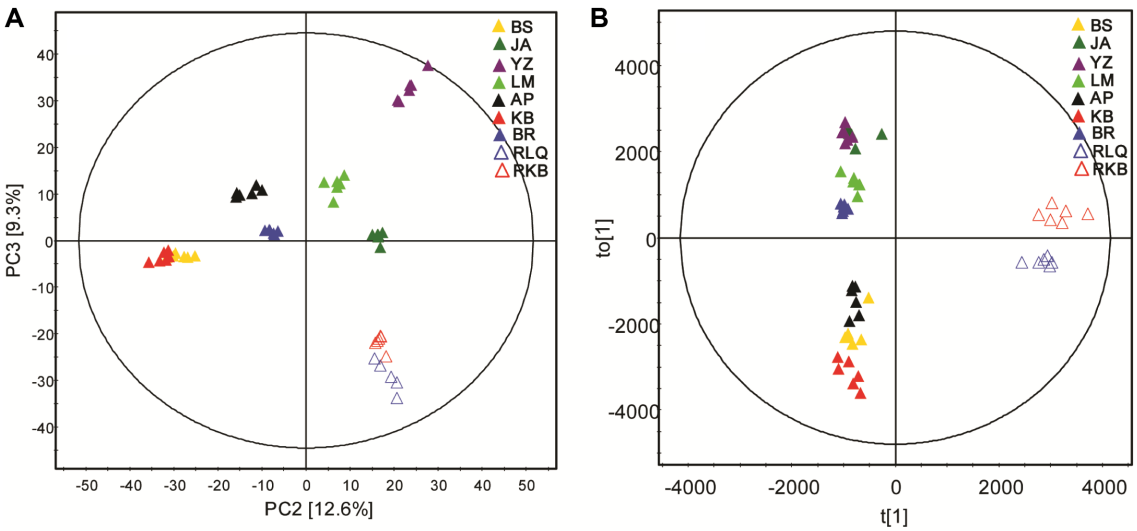

Fig. 2. Principal component analysis score plots (**A**) and orthogonal partial least square discriminant analysis score plots (**B**) from gas chromatography time-of-flight mass spectrometry analysis of commercial vinegars (BS, JA, YZ, LM, AP, KB, and BR) and traditional vinegars (RKB and RLQ).

▲ Balsamic vinegar (BS); ▲ Japanese apricot vinegar (JA); ▲ Yuzu vinegar (YZ); ▲ Lemon vinegar (LM); ▲ Apple vinegar (AP); ▲ Korean Black raspberry vinegar (KB); ▲ Brown rice vinegar (BR); △ Rural lacquer vinegar (RLQ); △ Rural Korean Black raspberry vinegar (RKB).

Table 2. List of significantly different primary metabolites between commercial vinegars and traditional vinegars as identified by GC-TOF-MS.

No.[a]	Rt (min)[b]	Compound[c]	MS fragment ions (*m/z*)	TMS[d]	*p*-value	ID[e]
			Sugars & sugar alcohols			
1	10.70	Xylitol	217, 147, 133, 129, 75	TMS (X5)	1.18E-09	std
2	11.96	Fructose	217, 147, 133, 117, 103	TMS (X5)	2.48E-08	std
3	12.20	Glucose	204, 160, 133, 117, 103	TMS (X5)	5.69E-07	std
4	12.34	Sorbitol	205, 160, 147, 117, 103	TMS (X5)	5.12E-03	std
5	12.94	Glucopyranose	217, 205, 204, 191, 129	TMS (X5)	9.48E-05	std
6	13.33	*myo*- Inositol	305, 217, 191, 147, 103	TMS (X6)	1.24E-06	std
			Organic acids			
7	7.30	Succinic acid	149, 148, 75, 74, 59, 55	TMS (X2)	3.08E-06	std
8	8.94	Malic acid	148, 133, 117, 75, 59	TMS (X3)	1.33E-06	std
9	11.50	Citric acid	273, 211, 183, 149, 147	TMS (X4)	4.56E-04	std
			Others			
10	6.96	Glycerol	205, 148, 147, 133, 117	TMS (X3)	3.80E-02	std
11	7.34	Ethanolamine	175, 147, 133, 100, 89	TMS (X3)	3.08E-06	std

Variables were selected based on variable importance in projection (VIP >0.7) and *p*-value (<0.05).

[a]Number of metabolite.

[b]Retention time.

[c]Identified: standard mass spectrum was consistent with those of standard compounds.

[d]TMS, trimethylsilyl.

[e]Identification: std, standard compound.

levels were high in commercial vinegars, whereas xylitol, *myo*-inositol, succinic acid, glycerol, and ethanolamine levels were high in traditional vinegars.

Antioxidant Activity, Total Flavonoid Content, and Total Polyphenol Content of Various Vinegars

The results of the three *in vitro* assays (ABTS, DPPH, and FRAP), total flavonoid contents, and total polyphenol contents are presented in Fig. 4. The ABTS (Fig. 4A) value of RKB was highest at 0.93 ± 0.004 μmol trolox equivalents (TE)/g, followed by RLQ and JA. DPPH (Fig. 4B) and FRAP (Fig. 4C) also appeared to have a similar pattern and had a similar tendency. The estimated total flavonoid and total polyphenol contents of the nine vinegars are presented in Figs. 4D and 4E. The total flavonoid content of various vinegars ranged from 1.54 to 182.42 ppm (Fig. 4D). Of the vinegars, the total flavonoid content of RKB was the highest at 182.42 ± 1.823 ppm, followed by 43.47 ± 0.526 ppm for YZ, and 39.44 ± 2.596 ppm and 33.30 ± 1.324 ppm for RLQ and JA, respectively. Except for YZ, the result of antioxidant activity and total flavonoid contents showed a similar tendency. The total polyphenol contents of vinegars ranged from -4.55 to 276.62 ppm (Fig. 4E). Among the vinegars, RKB had the highest total polyphenol content at 276.62 ± 1.141 ppm, followed by 213.58 ± 1.848 ppm for RLQ, and 67.91 ± 1.730 ppm and 61.77 ± 2.685 ppm for JA and BS, respectively.

Target Analysis of Compounds Associated with Antioxidant Activity Using UPLC-Q-TOF-MS and LC-IT-MS/MS

UPLC-Q-TOF-MS and LC-IT-MS/MS were used for target analysis to detect secondary metabolites associated with antioxidant activity. The molecular weights of the antioxidant compounds carotenoids (beta-carotene, lycopene, and lutein) and flavonoids (anthocyanins, catechins, quercetins, and proanthocyanidins) in the various vinegar samples were determined. Cyanidin-3-xylosylrutinoside (a), cyanidin-3-rutinoside (b), and quercetin (c) were detected (Table 3) and, those metabolites were found mainly in RKB compared with other vinegars (Figs. 5 and S1).

Discussion

In this study, non-target and target metabolite profiles of seven commercial vinegars and two traditional vinegars were determined by GC-TOF-MS and UPLC-Q-TOF-MS

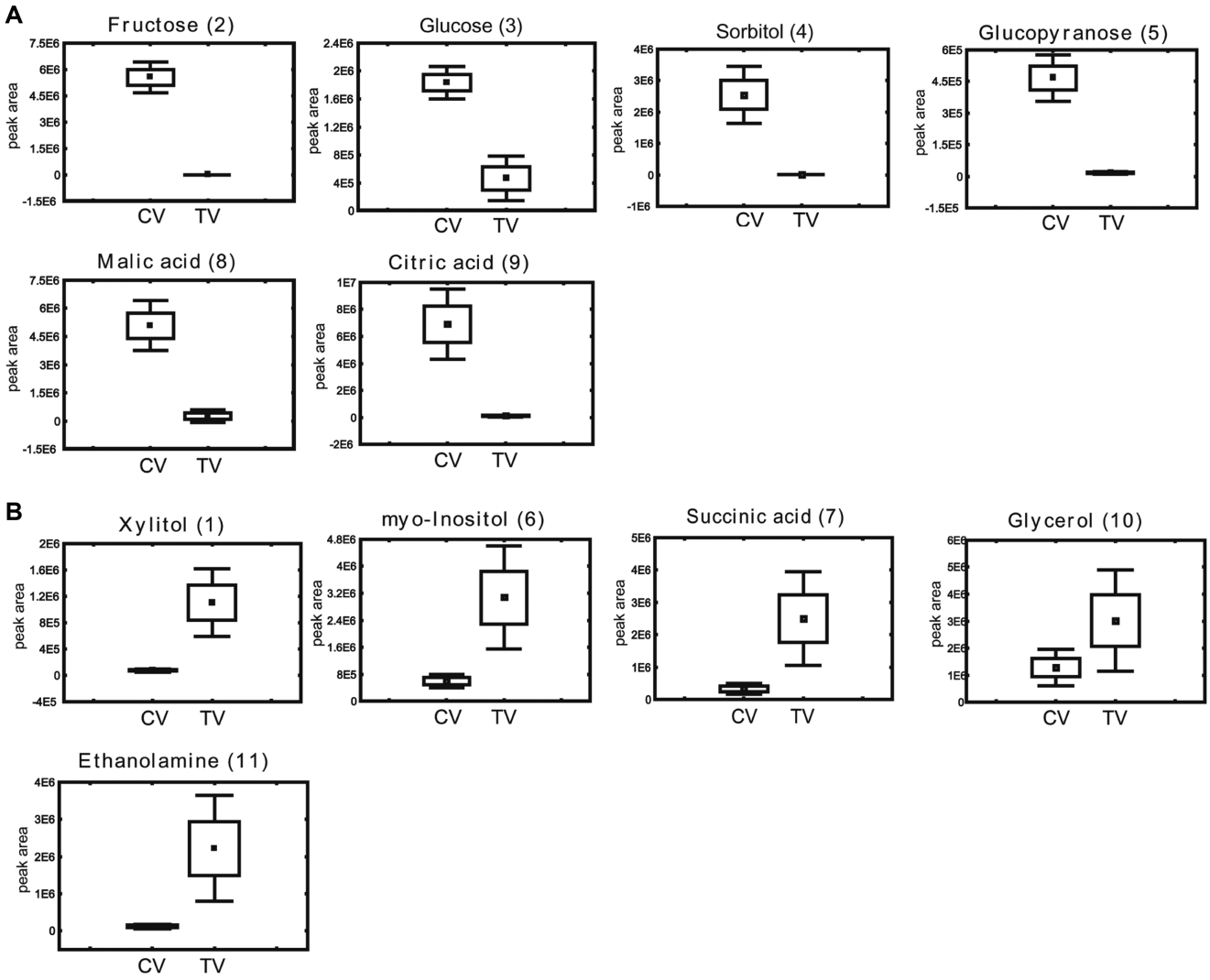

Fig. 3. Box-and-whisker plots of primary metabolites.

The plots varied significantly between commercial vinegar and traditional vinegar: higher content in commercial vinegars than traditional vinegars (**A**) and higher content in traditional vinegars than commercial vinegars (**B**). CV, commercial vinegars (BS, Balsamic vinegar; JA, Japanese apricot vinegar; YZ, Yuzu vinegar; LM, Lemon vinegar; AP, Apple vinegar; KB, Korean Black raspberry vinegar; and BR, Brown rice vinegar); TV, traditional vinegars (RLQ, Rural lacquer vinegar; and RKB, Rural Korean Black raspberry vinegar).

with multivariate analysis. As shown in Fig. 3, we thought artificial sweeteners and saccharide were added to the commercial vinegars during the acetic acid fermentation process. That is why the saccharide content of commercial vinegars was higher than in the traditional vinegars (Fig. 3). According to Park *et al*. [25, 28], during alcohol fermentation, yeast, *nuruk*, and *koji* were used as sugars for nutrients and as the fermentation substrate that was used for the biochemical saccharification of starch to alcohol. It seems that the sugar content of traditional vinegars decreased and the alcohol percentage increased during fermentation processing.

In this study, the ABTS, DPPH, and FRAP results were similar to those of total flavonoid content and total polyphenol content (Fig. 4), except for YZ and RLQ. YZ is a popular citrus fruit in Korea and Japan, having flavonoids and vitamin C in its peel [14], and RLQ is made using *Rhus verniciflua* stokes extracts that include phenolic compounds [16]. For these reasons, the total flavonoid content of YZ and total polyphenol content of RLQ were shown to be high.

Among the nine vinegars, several polyphenol compounds, such as cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside,

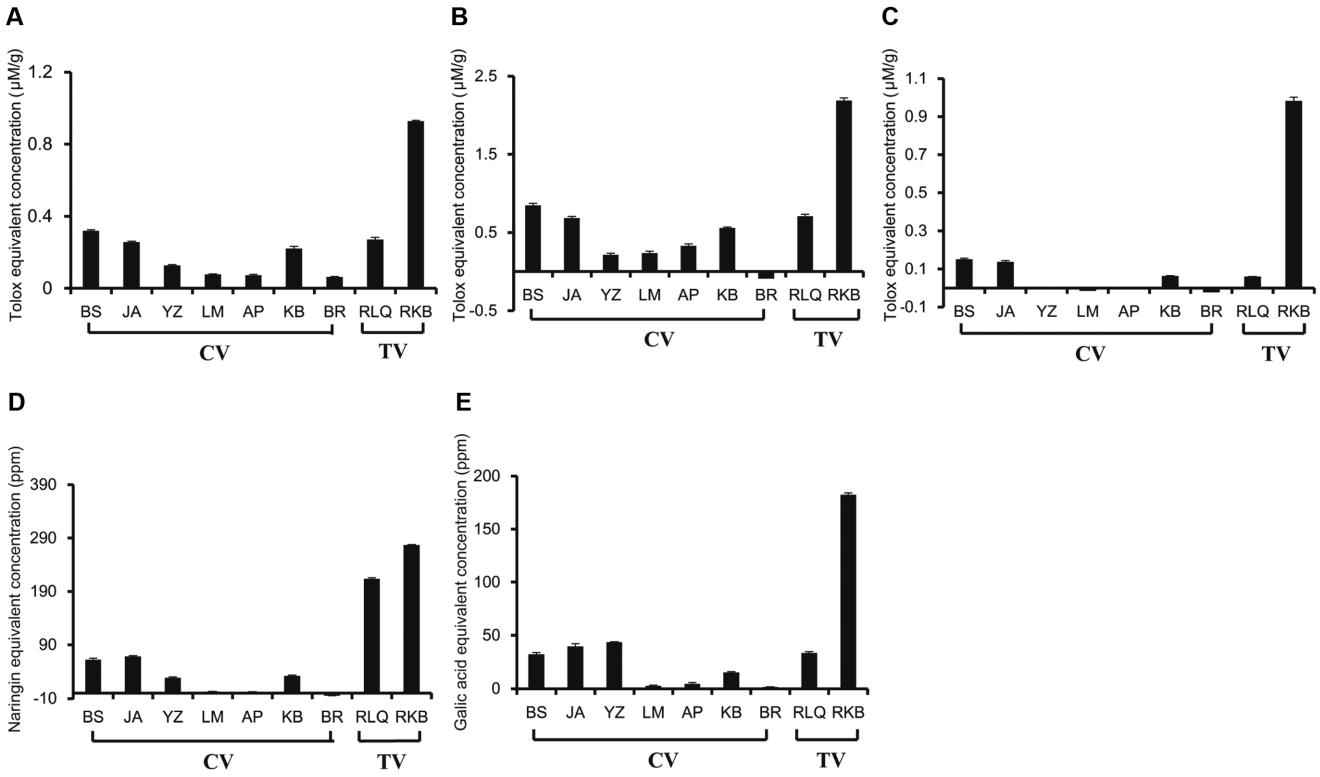

Fig. 4. Antioxidant activity of vinegar samples: ABTS (**A**), DPPH (**B**), FRAP (**C**), TPC (**D**), and TFC (**E**) of vinegars.
CV, commercial vinegars (BS, Balsamic vinegar; JA, Japanese apricot vinegar; YZ, Yuzu vinegar; LM, Lemon vinegar; AP, Apple vinegar; KB, Korean Black raspberry vinegar; and BR, Brown rice vinegar); TV, traditional vinegars (RLQ, Rural lacquer vinegar, and RKB, Rural Korean Black raspberry vinegar). All experiments were performed in triplicate.

Table 3. Target metabolite analysis using UPLC-Q-TOF-MS and LC-IT-MS/MS associated with antioxidant activity.

Tentative metabolites[a]	Rt (min)[b]	UPLC-Q-TOF-MS				LC-IT-MS/MS		ID[c]
		Formula	ppm	Measured mass (*m/z*)		MS fragment ions (*m/z*)	UV (nm)	
				$[M-H]^-$	$[M-H]^+$			
Cyanidin-3-xylosylrutinoside (a)[d]	3.03	$C_{32}H_{39}O_{19}$	-2.1	725.1891	727.2053	727>287>213	276, 591	ref [30]
Cyanidin-3-rutinoside (b)	3.09	$C_{27}H_{31}O_{15}$	-1.9	593.1469	595.1700	595>287>213	278, 518	ref [30]
Quercetin (c)	5.07	$C_{15}H_{10}O_7$	0.3	301.0356	303.0430	-	-	std

[a]Tentative metabolites based on variable importance projection (VIP) analysis with a cutoff value of 0.7 and a p-value < 0.05.
[b]Retention time (min).
[c]Identification: std, standard compound; ref, references.
[d]Letter of metabolite.

and quercetin were detected at high levels in RKB (Table 3, Fig. 4).

Polyphenol compounds are secondary metabolites that have been shown to have high levels of antioxidant activity [29]. Berries, including black raspberry, are well known to contain high levels of quercetin and cyanidin, which are antihypertensive, anti-inflammatory, and anticancer [4, 5] antioxidants, and have an influence on immune activities and cardiovascular diseases [6, 32]. According to Hassimotto *et al.* [9], anthocyanin-rich fruits and vegetables appear to have higher levels of TPC and TEAC, which accounts for the high antioxidant activity (ABTS, DPPH, and FRAP). Therefore, since RKB contained high contents of the metabolites associated with antioxidant activity (Fig. 5), RKB was shown to have high antioxidant activity.

In our current study, 11 primary metabolites, including

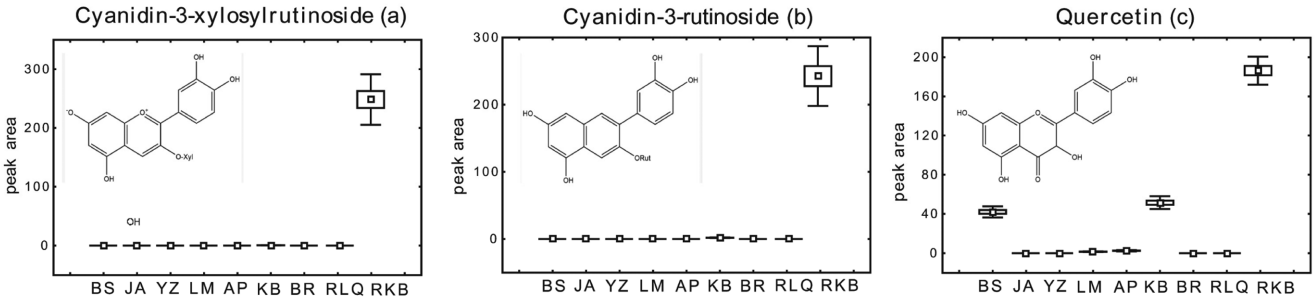

Fig. 5. Box-and-whisker plots of target analyzed secondary metabolites related with the antioxidant activity.

sugars and sugar alcohols, amino acids, and others, were detected as being significantly different between commercial and traditional vinegars using GC-TOF-MS-based non-target MS profiling. The pH, total acidity, antioxidant activity, total flavonoid content, and total polyphenol content were also measured. The results for the pH and total acidity conformed to the classification of the Korean food standard code. In the antioxidant activity assay, RKB measured the highest ABTS, DPPH, FRAP, total flavonoid content, and total polyphenol content among the nine vinegars. However, YZ and RLQ showed high total flavonoid content and total polyphenol content owing to the characteristics of each material. Target analysis of the secondary metabolites using UPLC-Q-TOF-MS showed association with antioxidant activity. Cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside, and quercetin were detected, and those compounds were more highly detected in RKB, than in the other vinegars. We believe those kinds of polyphenols and flavonoids contributed to the high antioxidant activity in RKB.

In this study, we investigated the metabolite differences between commercial and traditional vinegars using MS-based metabolite profiling. We also identified three secondary metabolites, which were associated with the antioxidant activities of the vinegars, by target analysis using UPLC-Q-TOF-MS. Our study suggests that MS-based metabolite profiling can be a useful tool for evaluating the quality of vinegar. However, further studies of metabolite profiling of vinegars taking into account the fermentation periods and differences in fermentation processing methods are needed to improve the knowledge of the vinegar manufacturing industry.

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